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Short Membrane Proteins from Viruses: Channel-Pore Dualism?

George Patargias[1], Hugo Martay[1], Jens Krüger[2], Chin-Pei Chen[2], Yi-Ting Wang[2], Ming-Da Lee[2], Cheng-Chang Chen[2], Guo-Sheng Hong[2], and Wolfgang B. Fischer[2]

[1] Department of Biochemistry, University of Oxford, Oxford OX2 3QU, UK

[2] Institute of Biophotonics, School of Biomedical Science and Engineering, National Yang-Ming Univeristy, Taipei 112, Taiwan
E-mail: wfischer@ym.edu.tw

The genomes of some viruses encode small membrane proteins which are known to alter membrane permeability by forming ion conducting pores. The alteration of the electrochemical gradient as a consequence of 'channel activity' has large scale consequences as it induces the fusion and budding process of the virion. Viral channel or pore forming proteins exist with different numbers of transmembrane (TM) domains. The working hypothesis is that the proteins diffuse as monomers in the lipid membrane and finally self-assemble to form the functional unit. Self-assembly has to take place at the level of the tertiary and quaternary structures within the low dielectric medium of the lipid membrane. Computational techniques are used to analyze the assembly process. Ion flux is simulated using steered molecular dynamics (MD) simulations and analyzed using Langevin equation of motion. Conductance measurements flank the in silico investigations. Data of the channel forming protein Vpu from HIV-1 will be shown as a test case.

1 Introduction

In a series of viruses short membrane proteins have been identified which are found to alter permeability of host cell membranes[1]. For some of them such as M2 from Influenza A, functional analysis have been done and it is now established that M2 acts as a proton channel. For others proteins such as Vpu from HIV-1, p7 from HCV, and others data on ion conductance were obtained by reconstituting the proteins into artificial bilayers. In analogy to M2 however it is concluded that these proteins act as channel forming proteins in vitro as well. For Vpu from HIV-1 for example increasing evidence is now given in the literature that the protein interacts with host cell factors and hampers their mode of action. In other words besides altering the permeability of the lipid membrane Vpu acts also as a cellular modulator via assembling with host cell proteins. No matter which role the proteins accomplish they have to assemble to fulfill their role in the life cycle of the virus either with themselves to form channels or pores or with host cell factors. In the light of these findings on a molecular level the question arises (i) once assembled, how selective are these proteins, and (ii) what is the mechanism of assembling per se. These questions are also relevant for other diffusion processes across the lipid membrane such as viral fusion, DNA/RNA delivery and even budding. Structural information of these proteins is emerging via NMR spectroscopic investigations. For Vpu they have confirmed that the TM domain is helical. Computational methods may serve as a torch to enlighten atomic details, since high resolution data is not available for all of the proteins.

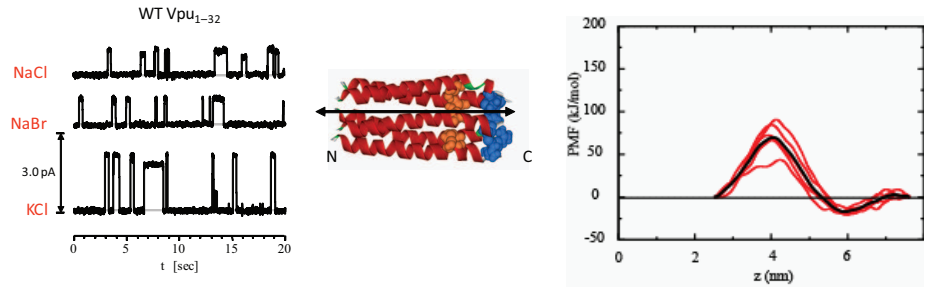

Figure 1. Bilayer recordings of Vpu_{1-32} using different types of salt at a holding potential of 100 mV, 500 mM salt concentration, HEPES, pH 7, reconstituted into POPE : DOPC (1:4) (left). Pentameric model of Vpu_{1-32} (middle) through which the ions are pulled. Respective potential of mean force of a K-ion being pulled through the pore. In black the averaged values for 5 (red) individual pullings.

2 Results

2.1 Experiments

Experimental findings with a peptide containing the first 32 amino acids of Vpu, Vpu_{1-32} (MQPIPIVAIV$_{10}$ ALVVAIIIAI$_{20}$ VVWSIVIIEY$_{30}$ RKI), reconstituted into artificial lipid bilayers show conductance in the range of 17 - 20 pS in 300 - 500 mM KCl solution[2]. When changing the anion, e.g. NaCl to NaBr a small change in conductance is found compared to a larger change when changing from NaCl to KCl. This indicates that the assembled bundle prefers to conduct cations over anions. However in the light of conductance measurements with a series of other monovalent chloride salts it is concluded that the bundle is only a weakly selective channel. Together with the findings of altered substrate permeability by other groups it is concluded that the bundle assembly may be able to conduct ions AND small molecules. The mechanism of how the bundle discriminates is still not clear. It is suggested, that conformational changes due to lipid composition are responsible.

2.2 Computer Simulations

To transfer the experimental data into in silico experiments helical models of a pentameric assembly of Vpu_{1-32} have been generated using XPLOR (A. Bruenger). Using a helical motif for the TM domain is suitable based on NMR experiments. The bundle model has a hydrophilic C terminus due to Ser-24 and Arg-31 facing the lumen of the pore and a hydrophobic stretch towards the N terminus. With steered molecular dynamics simulations in a fully hydrated lipid bilayer using Gromacs 3.2.1 (Gromos 96 ff, PME) K-, Na- and Cl-ions have been pulled through the lumen of the pore. Assuming a model of one-dimensional stochastic dynamics for the ion Langevin equation is used to calculate the potential of mean force of the ion. The data indicate a s-shaped curve with a minimum at the C terminus and a maximum within the pore in the region of the hydrophobic stretch produced by the amino acids towards the N terminus. A comparison of the energy bariers of the different ions shows a preference for cations over the anion. Based on the current

model and physical method to calculate the PMF the in silico data support the experimental data of Vpu being a weak channel.

3 Discussion

The quality of the current data depends on the physical model used for ion diffusion, the method used to derive the relevant data and the bundle model itself. To elucidate the affect of the bundle emphasis is currently given to establish a protocol for generating reliable bundle models. The approach can be summarized as following: (i) detection of the TM domain(s) of the protein using secondary structure prediction programs, (ii) obtaining an equilibrated structural model of the momoner[3], and (iii) generating high quality bundle models by screening conformational space. In this last stage a protocol is used which combines backbone positioning followed by side chain generation (Krüger and Fischer in this Proceedings p. 269). All the calculations are based on the assumption that the protein is produced as a monomeric unit in the endoplasmic reticulum and will fold and diffuse as a monomer prior to assembly.

4 Concluding Remarks

Self-assembly of the Vpu protein leads to a weak selective bundle, which may be also true for other viral proteins with a single TM domain. Depending on the experimental or in vivo conditions Vpu may also allow substrates to pass (channel-pore dualism). Correlation of in silico models with experimental data can be achieved to elaborate on the structure-function paradigm. Calculating functional data will be used to suggest the most reliable models.

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References

1. W. B. Fischer, *Protein Reviews: Viral membrane proteins: structure, function and drug design. Volume 1* (Kluwer Academic / Plenum Publisher, New York, NY, 2005).
2. T. Mehnert, A. Routh, P. J. Judge, Y. H. Lam, D. Fischer, A. Watts and W. B. Fischer, *Biophysical characterisation of Vpu from HIV-1 suggests a channel-pore dualism.*, Proteins **70**, 1488-1497, 2008.
3. J. Krüger, and W. B. Fischer, *Exploring the conformational space of Vpu from HIV-1: a versatile and adaptable protein.*, J. Comp. Chem., in print, 2008.